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Chapter V

FUNCTIONAL REGENERATION IN THE OPTIC SYSTEM

By

R. W. SPERRY

THE following discussion is divided into two sections. The first is a brief survey of what is known about the capacity of the optic nerve (*fasciculus opticus*) to regenerate in the main classes of vertebrates. This is followed by some comments on the quality of functional recovery where it occurs and a consideration of the determining factors.

CAPACITY FOR FUNCTIONAL REGENERATION

Mammals: The mammals may be dispensed with rather quickly because the evidence to date indicates that ordinarily vision is never recovered by regeneration of the optic nerve in this group (Tello, '07; Rossi, '09; '12; Rossi and Gastaldi, '35; Stone, '38; Polyak, '41; Eayrs, '52).[1] As a rule, interruption of the optic axons anywhere along their course, even at their termination in the lateral geniculate nucleus, is followed by a retrograde degeneration that extends peripherally into the retina to include the ganglion cells. In this connection it is interesting that, except in early youth, degeneration of the optic axons does not follow retrograde degeneration of the cells of the lateral geniculate nucleus (Polyak, '41).

The histological effects of intracranial transection of the optic nerve in young rabbits less than a month old were reported in some detail by Rossi ('12). He described considerable sprouting and local outgrowth of fibers in the retina and in the attached peripheral nerve stump but these fibers did not succeed in crossing

[1] Poscharissky ('09) noted slowly degenerating fibers which might be mistaken for regeneration in severed optic nerves of puppies and wolf cubs. *Ed.*

the nerve scar to re-establish functional connections in the brain.

Early reports of visual recovery following transplantation or reimplantation of eyes in the rat (Koppanyi, '23a; Koppanyi and Baker, '25) have not been corroborated in subsequent studies (Keeler, '29; Matthews, '33; Stone, '30; '38).[1] Guist ('26) found that visual recovery was not possible in the rat if the blood supply was cut off from the retina for from 15 to 30 minutes.

I once divided the optic nerve in 18 infant rats (1941, unpublished) by merely crushing the nerve between forceps through a dorsolateral incision into the orbit. This left the artery and the dural sheath of the nerve intact but divided completely the neural core of the nerve. The entire procedure was visualized through a stereoscopic microscope. After crushing, the nerve stumps could be seen within the intact translucent sheath separated by less than 0.5 mm. Two to three months later the eye showed varying degrees of underdevelopment, having reached in the best few cases only approximately three-fourths its normal size. Visual reactions were absent and later examination revealed in the place of the optic nerve only a thin connective tissue strand apparently devoid of optic fibers. Much the same results, including histological observations, have been reported recently by Eayrs ('52).

The geniculostriate system appears to be equally lacking in regenerative vigor. Retrograde degeneration is the general rule following division of the radiation fibers including merely interruption of their cortical terminals (Polyak, '41). Fibers ending only a few microns away from a cortical lesion, however, may remain intact.

Birds and Reptiles: The birds and reptiles also may be passed over quickly, not because of any well-demonstrated absence of regenerative capacity, but merely for lack of evidence one way or the other. A comparison of the results of optic nerve section in reptiles during winter hibernation and in midsummer might be informative.[2]

[1]See also Collevati ('26) who believed some reflexes returned after transplanting rats' eyes. *Ed.*

[2]Central nervous regeneration was studied in the dormant adder as well as the hibernating dormouse to a limited extent by Rossi ('10a) and his pupil, Zalla ('10). *Ed.*

Fishes: The picture in the fishes and amphibians stands in marked contrast to that in the mammal. Among the fishes the ability of the optic nerve to regenerate and to restore vision after its complete transection seems to be widespread. All forms we have investigated have shown good visual recovery. This includes 7 species, 5 marine (Sperry, '48b) and 2 fresh water (unpublished data on *Gambusia holbrooki* and *Betta splendens*) from 5 separate families. Recovery in another distant fresh water form has been reported by Rasquin ('49).

It is important to emphasize that all the specimens we have used have been under 10 cm in length. It is entirely possible that functional recovery would be less satisfactory in the larger fishes. Onset of visual recovery has been seen as early as 6 days after nerve transection in small marine teleosts two centimeters in length (Sperry, '48b). In our limited experience the marine species have recovered more rapidly and thoroughly than the fresh water forms. Matthews ('33) reported a lack of visual recovery after optic nerve section in 4- to 6-cm *Fundulus heterolitus* but the absence of retrograde degeneration and the extensive neuroma formation he described suggest considerable regenerative vigor in the nerve.

If the blood supply to the eye is interrupted in transecting the optic nerve the lens and retina generally undergo degeneration before circulation is re-established and these structures very seldom, if ever, regenerate to a functional level in the fishes (Ask and Andersson, '33; Matthews, '33; Stone, '40; '41). Attempts to transplant or to reimplant the eye have been negative for the most part so far as visual recovery is concerned. The grafted eyes frequently take and may appear quite normal for the first few weeks but then they undergo gradual regressive changes (Stone, '40; '41). In only a few rare instances among hundreds of grafted eyes has visual recovery been reported. Koppanyi ('23a) mentions a recovery of light sensitivity in fishes. Kolmer ('23) conducted some histological studies on these preparations. More recently Rasquin ('49) described good recovery of visual feeding reactions through both reimplanted eyes of a single specimen of *Astyanax mexicanus* as early as 5 weeks after operation. In 8 other specimens, similarly operated, recovery failed to occur in either eye. It would

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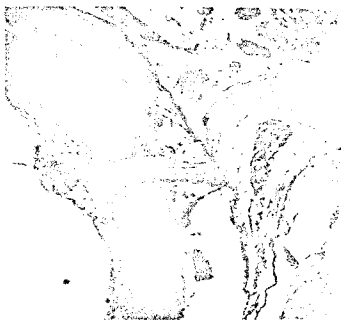

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A few years ago I was able to get good visual recovery in 7 out of 16 reimplanted eyes using the marine teleost, *Bathygobius soporator* (Sperry, '49). One week after the onset of recovery these fish were localizing accurately small 3-mm objects at a distance of 10 cm. These results are atypical, however, and can

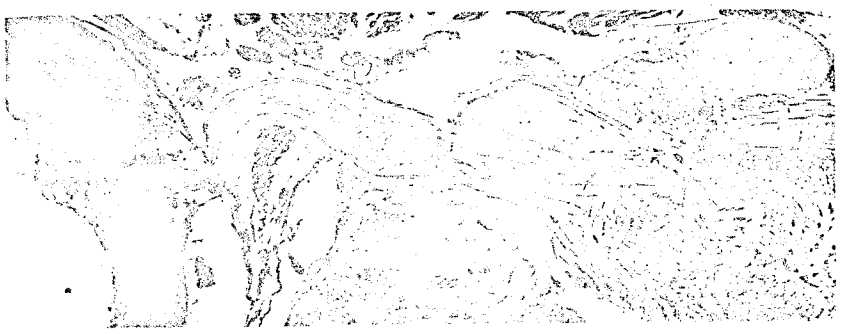

FIGURE 24. Photomicrograph of an oblique transverse section from a fish (*Bathygobius soporator*) that recovered vision after reimplantation of eye. The regenerated optic nerve can be traced through almost its entire course from retina to optic lobe. Optic fibers are sparse compared to a normal nerve. Partial patchy degeneration of the retina was evident particularly in the central area. Bodian protargol method.

be ascribed to the following: (1) selection of a hardy tidepool species physiologically adapted to withstand low oxygen tensions; (2) use of small and young specimens only 1.5 to 2.0 cm in length; (3) use of the free external cornea possessed by this species to hold the reimplanted eye in position, and (4) placing the fish in well-aerated, running whole sea water in a darkroom immediately after operation on the supposition that retinal metabolism might be somewhat reduced in darkness. Which of the foregoing factors are critical has never been determined. The regenerated optic fibers were much fewer than in the normal nerve and patches of retinal degeneration were evident in all cases (Fig. 24).

Urodele Amphibians: The urodeles have been more extensively studied than any of the other vertebrates, probably in part because of the greater success in obtaining visual recovery, especially after eye transplantation. In a long series of experiments that extends back into the latter third of the eighteenth century (for leading references see Keeler, '29; Mangold, '31; Matthews, '33; Matthey, '25; '26a, b; Reyer, '48; Stone, '30; '50) it has been shown that the transected optic nerve regenerates readily in both larval and adult urodeles to bring good visual recovery. More than this, an entire new retina can be regenerated in the adult following degeneration of the old retina as occurs after temporary interruption of the blood supply in eye transplantation and sometimes in optic nerve section. The newly regenerated retina sprouts a new optic nerve that generally connects with the brain to bring good recovery of vision.

In his extensive studies of transplanted eyes Stone has been able to show further that the adult *Triturus* eye can be transplanted as many as 4 times with recovery of vision in each of the new hosts (Stone and Farthing, '42) and that vision can be recovered after the eyes have been refrigerated for as long as 7 days before transplantation (Stone, '46). He also has demonstrated visual recovery after transplanting eyes between two different species of *Amblystoma* (Stone, '30).

Finally, it has been found that the secondary intracentral fiber tracts that link the mesencephalic optic lobe with the lower bulbar and spinal centers are capable of functional regeneration following their transection in adult *T. viridescens* (Sperry, '48a). The transections were made just anterior to the cerebellum and involved all fiber tracts passing through this level. Specific functional tests were run only for the optic system but presumably function was restored in the other fiber systems as well, judging from the gradual disappearance of nearly all of the early postoperative abnormalities in posture and movement.

Anuran Amphibians: In the early 1920's Koppanyi ('23) included frogs and toads among the various vertebrates in which he had seen recovery of vision after eye transplantation. Failure to confirm these findings in subsequent studies led to a growing

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conviction that the optic nerve of frogs and toads including their tadpole stages, lacks the power to regenerate (Cole, '25; Keeler, '29; Stone, '40; '41). This inference was based largely on the fact that the retina frequently survived in apparently excellent condition in grafted eyes but failed to form an optic nerve.

We have since discovered that if the nerve is transected without interrupting the blood supply excellent regeneration and recovery of vision is obtained in both larval and adult anurans of many species including representatives of the families *Hylidae*, *Ranidae* and *Bufonidae* (Sperry, '44; '45a). It is my impression that the capacity for functional regeneration tends to fall off in the full-grown adults of the largest varieties of anurans particularly among the *Ranidae*.

Having established that the anuran optic nerve is capable of good functional regeneration under favorable conditions, we made another attempt to transplant the eye. As in earlier investigations no visual recovery was found in tadpole stages (14 cases) but good restoration of vision was obtained in 2 out of 16 transplants in young frogs that were in the process of metamorphosis (Sperry, '45a). The eye undergoes rapid enlargement at this time and apparently the retina and optic nerve have a higher potential for survival and regeneration than at earlier or later stages. It should be possible to get a higher percentage of successful recovery if care were taken to oppose the nerve stumps and to properly orient the eyeball. In these experiments the eyes had been transplanted to the opposite orbit and inverted on the dorsoventral axis.

QUALITY OF THE RECOVERED VISION

The problems involved in obtaining orderly functional recovery after regeneration in the optic system illustrate the kind of thing one must deal with wherever the question of functional restoration is met following regeneration in the central nervous system.

No attempt has yet been made to test systematically either visual acuity or brightness discrimination following optic nerve regeneration. It seems highly probable, owing to the loss of optic fibers

and their failure to find adequate central terminations, that the restoration of acuity is below normal in varying degrees. I have seen tree frogs with recovered vision leap accurately to catch house flies at a distance of 40 cm and similarly fish at a distance of 20 cm would turn and swim directly at minute crumbs of food no longer than one millimeter which were slowly sinking in the water. The results in general indicate that the recovered visual acuity may approach a normal level under favorable conditions.

Tests of color perception after optic nerve regeneration have not been described in the literature. A few years ago we found that fishes (*Gambusia holbrooki*) with regenerated optic nerves would feed from an orange-colored ring suspended in the water and would avoid similar rings colored black, white or gray suspended along side after cues other than color such as position, pattern, odor, etc., had been ruled out (Sperry and Deupree, unpublished). The color discrimination had been trained before optic nerve section and apparently did not have to be relearned after regeneration. Based on only 3 cases that survived with recovered vision, these findings require further investigation.

More conclusive results have been obtained with reference to optokinetic reactions, the localization of small objects in space and the perception of the direction of movement of small objects (Sperry, '42; '43a, b; '44, '45a; '48b; '49; '51a, b; Stone, '48). All show that the spatial local sign properties of points in the retinal field are restored in an orderly manner. The recovered visuomotor co-ordinations are quite normal provided the eye is normally oriented in the orbit and the regenerated fibers connect with the correct side of the brain. If, on the other hand, the eye is rotated 180° in its orbit the animals behave as if everything in the visual field appeared upside down and reversed front-to-back. If the eye is transplanted to the opposite orbit with only one axis inverted the animals respond as if the visual field were reversed on only the one axis. If the optic nerves connect to the wrong side of the brain, objects to one side of the midline are falsely localized to a corresponding point on the opposite side. Combinations of such anatomical rearrangements yield intermediate forms of visual behavior correlated directly with the anatomical disarrangements.

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We find that the foregoing applies in all cases where vision is restored after regeneration in the optic system, i. e., in fishes and in urodole and anuran amphibians, in both larval and adult stages, and whether the original nerve has regenerated directly or a new nerve has formed from a newly regenerated retina. It is as if optic axons arising from any point in the retinal field possess an intrinsic predilection to form their central functional relations with neurons in a matching locus of the optic tectum.

It has been concluded from the results that the optic fibers must differ from one another in quality as must also the central neurons of the optic tectum on which they terminate. We have suggested that the required specificity of the optic axons and tectal neurons could be achieved through a polarized differentiation of the retinal and tectal fields in development. The retinal differentiation would have to proceed on at least two separate axes in order to stamp the neurons of every retinal locus with unique properties. The same applies to differentiation of the tectum.

The orderly recovery of retinal local sign can then be accounted for on the basis of selective central synapsis regulated by specific chemoaffinities between neurons of matching spots in the retinal and tectal fields. One does not picture a rigid point-to-point projection in these lower vertebrates but rather a point-to-focal area projection with considerable overlap in the terminal areas of neighboring retinal fibers. Presumably the regenerating optic fibers undergo extensive arborization within the tectum. Also the dendrites of the tectal neurons themselves spread widely (Herrick, '17) to further favor the possibility of appropriate contacts. One pictures numerous contacts being made by each ingrowing fiber with only the appropriate ones being reinforced and maintained in a functional state by the specific interneuronal affinities. Possibly numerous terminal branches which fail to establish appropriate connections undergo atrophy and resorption. In general the proper patterning of synaptic relations in normal development is thought to proceed on much the same basis as pictured here for regeneration, not in the visual pathways alone but throughout the nervous system (Sperry, '51a, b).

Local lesions restricted to a particular quadrant of the optic lobe in the frog produce scotomata in corresponding quadrants

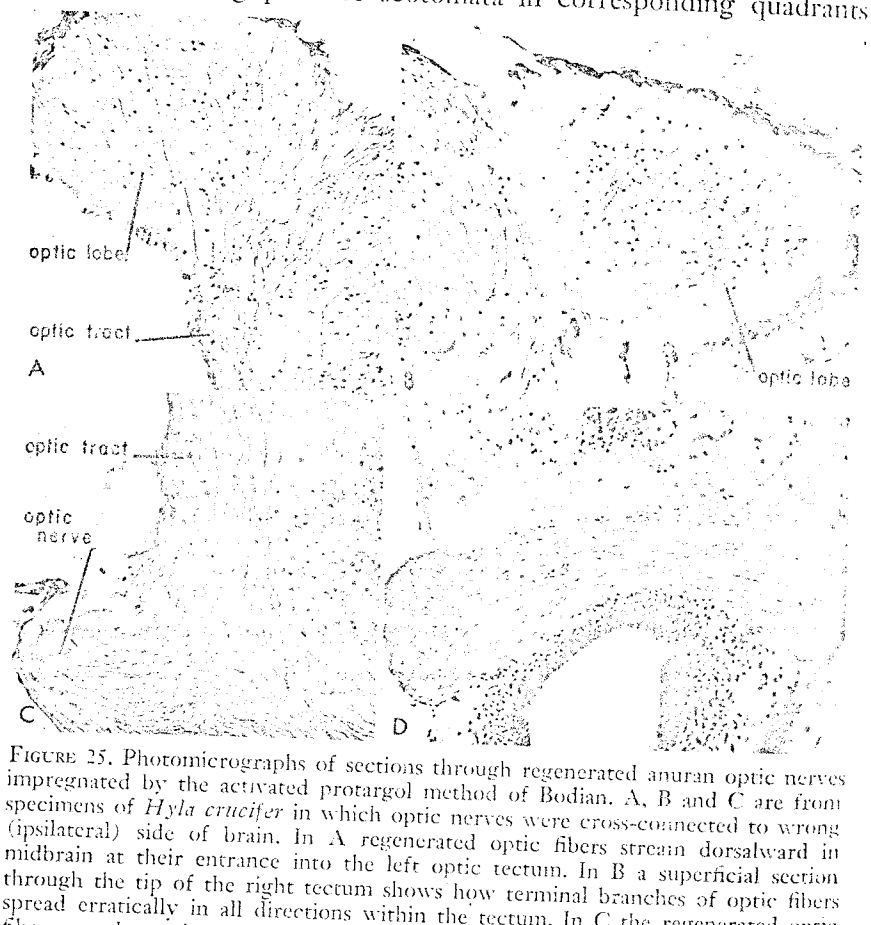

FIGURE 25. Photomicrographs of sections through regenerated anuran optic nerves impregnated by the activated protargol method of Bodian. A, B and C are from specimens of *Hyla crucifer* in which optic nerves were cross-connected to wrong (ipsilateral) side of brain. In A regenerated optic fibers stream dorsalward in midbrain at their entrance into the left optic tectum. In B a superficial section through the tip of the right tectum shows how terminal branches of optic fibers spread erratically in all directions within the tectum. In C the regenerated optic fibers are shown in higher magnification entering the brain at the lower left and turning directly dorsalward in an ipsilateral tract leading to the left optic lobe. D is a frontal section through the chiasma region of a frog (*Rana clamitans*) which recovered vision that was functionally inverted after transplantation of the right eye into the left orbit.

of the visual field. Similar lesions after optic nerve regeneration produce scotomata in the same sectors of the visual field (Sperry, '44)suggesting that the regenerating fibers do indeed re-establish their functional linkages with cells in the same locus of the optic lobe.

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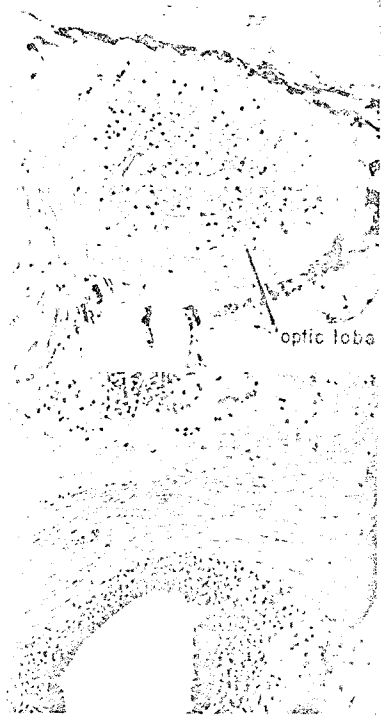

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In an unpublished study I found that lesions interrupting the medial optic tract in the normal frog (*H. cinerea*) produce a blind area in the dorsal sector of the visual field directly over the frog's head. Similar lesions after regeneration tended to be less effective in general and left fairly good vision in 4 to 7 cases in the same dorsal sectors. These preliminary findings suggest that the regenerating optic fibers destined for different quadrants of the tectum do not necessarily have to become segregated in the optic tracts before they re-enter the tectum. Fibers entering the tectum at its lateral border may make their way to terminations among cells of the medial quadrant. In line with this, the course of fibers in the outer optic stratum of the tectum after regeneration is not one of orderly parallel alignment. The fibers at any locus appear to run irregularly in all directions in a random network (Fig. 25B) .

The orderly restoration of visuomotor functions following regeneration of the tectobulbar and tectospinal tracts (Sperry, '48a) indicates that the fibers of these tracts and also the down-stream neurons in the bulb and cord on which they terminate are both subject to a high degree of qualitative specificity. If either the regenerating fibers or the downstream neurons were all alike and indistinguishable one from another there would be no way in which differential reflex relations could be re-established on an orderly plan, i.e., with functional readjustment eliminated as was shown by the results of eye rotation.

When added to the data on the optic nerve the findings on the tectospinal system along with similar observations on central nervous regeneration in the spinal dorsal roots (Miner and Sperry, '50; Sperry, '51b), in the trigeminal tracts (Miner and Sperry, '50; Sperry, '51a, b; Sperry and Miner, '49) and in the root and tracts of the vestibular nerve (Sperry, '45b; '51b) give further indication of the widespread nature of the phenomena illustrated above in the optic system (see also Weiss, '36; '41). If our interpretation of the data is correct an orderly recovery of normal function after central nervous regeneration generally requires a selective termination of specific fiber types on specific types of down-stream neurons. A possible exception might be in regions

where functional readjustment could compensate for abnormal synaptic relations. What evidence we have on this problem (Sperry, '45c) indicates that the central nervous system even of man is definitely limited, particularly in the subcortical regions, in its ability to correct for disarrangements of nerve fiber connections.

Chapter VI

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